Changes in the Morphological, Rheological, and Biochemical Blood Indicators in Triathletes

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Original article

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The aim of this study was to assess how the influence of intense physical effort changes the morphological, rheological, and biochemical blood indicators in triathletes. The study group comprised 10 triathletes aged 30-45 years, members of the Active Side of Life Association (Kraków, Poland). Venous blood was collected from the study participants twice, before and after the DiablakBeskid Extreme Triathlon 2016 (the Carpathians, Poland), and once from the control group for analysis of the selected blood indicators. Statistically significant changes were observed in the study group before and after the triathlon in morphological blood indicators, in the elongation index at the shear stress of 0.30 and 0.58 Pa, in levels of electrolytes, creatinine (mmol/l), serum protein parameters, and high-sensitivity troponin (ng/l). No such differences were reported for the remaining parameters. In turn, when comparing the study group before the triathlon with the control group, statistically significant differences were recorded in MCHC (g/dl), in the elongation index at the shear stress of 0.30 and 0.58 Pa, and Cl$^-$ (mmol/l) levels. No such differences were reported for the remaining parameters. Blood haematological and biochemical indicators in individuals that participate in triathlons characterize the actual range and direction of effort-related changes well and allow for the diagnosis of transient adaptive effects. Rheological parameters, involving the evaluation of erythrocyte deformability and aggregation, are useful for monitoring the particularly undesirable, short- and long-term effects of practicing extreme sports such as triathlons.

Key words: triathlon, blood, biochemistry, morphology, blood rheology.

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We are currently witnessing an advent of strongly individualized and sometimes surprising social preferences in choosing sports and recreational activities, increasingly characterized by the search for new, more exciting and extreme forms of expression (WALCZAK *et al.* 2011). Triathlons are a manifestation of this trend. Triathlons are one of the youngest sports, having emerged in the 1970s. They combine three races in different disciplines, one after another: swimming, cycling, and running. The growing popularity of running and marathons in the 1870s and 1880s encouraged many people to take up sports and live healthy lives. For those looking for new experiences, triathlons were the next natural step to take. The number of people that participate in triathlons has been constantly growing, among both professionals and amateurs. The discipline still raises some concern, but new areas are being discovered and boundaries of sports training crossed (KOSTYRA 2016). The first triathlon competition, combining three sport disciplines, i.e. swimming, cycling, and running, was held in San Diego, USA, in 1974. Jack Johnston and

Don Shanahan were the originators of the contest. The competition was organized in Mission Bay and involved 500 yards (457.2 m) of swimming, 5 miles (8 km) of cycling, and 6 miles (9.6 km) of running. About 50 competitors took part in the race. The first triathlon organization was established on August 17, 1984 in Almere, the Netherlands. It was the European Triathlon Union, with 10 member states in the year it was founded. In 1985, the first European Championship was held in Immenstadt, Germany, at the Olympic distance (1500 m swimming, 40 km cycling, 10 km running). In September 1989, the International Triathlon Union was founded in Avignon, France, and Les McDonald from Scotland became its president. Thanks to this organization, official world championships have been held since 1989 at the Olympic distance (BARSZOWSKI & KOSENDIAK 1999). In Poland, the first triathlon competition took place on July 14, 1984 in Kiekrz, near Poznań. The competitors had to cover 1.5 km of swimming, 50 km of cycling, and 20 km of running (BARSZOWSKI & KOSENDIAK 1999; KOSTYRA 2016).

Triathlons are an example of an extreme endurance sport. They require extensive aerobic effort, which leads to an increased risk of bodily harm, especially to muscles, and general inflammation. Physical endurance, i.e. the ability to delay the onset of fatigue and limit its effects, is an indispensable condition to cover the distance of a triathlon competition. The level of training of this skill determines to what extent weather conditions (wind, rain, high or low temperature, altitude difference, etc.) and the hardships of the competition will affect the athlete's body (MAŁEK *et al.* 2016). TULLOH *et al.* (2006) confirmed that triathletes faced similar medical problems, such as dehydration, hyperthermia, hyponatremia, hypothermia, skeletal muscle damage, and even myocardial damage. In turn, ROBERTS *et al.* (2016) highlighted triathletes' problems with gastrointestinal disorders, including diarrhea, abdominal pain, and nausea. It is estimated that up to 25-90% of athletes training endurance disciplines often pointed at these disorders as reasons for failure in competitions. MAŁEK *et al.* (2016) observed that triathlon training contributed to maintaining proper postural muscle length and increased mobility of the spine and chest. Triathletes are characterized by very well developed deep stabilizer muscles.

The type of workout load and weather conditions are important for changes in blood morphology in training athletes. Proper hydration during and after exercise prevents dehydration and thus plasma loss. This is particularly significant for physical efforts performed at high temperatures and high humidity (WIRNITZER & FAULHABER 2007). During exercise, blood volume decreases as a result of water loss when performing the thermoregulatory function and in or-

der to maintain blood oxygen potential with decreasing glycogen stores. The reduction in plasma volume is also due to the transfer of fluids between the extracellular and cellular compartments. As a result of the acid-base regulation, the plasma water is moved inside the exerted skeletal muscle cells. The restoration of morphotic parameters after a very intensive effort (ca. 90% VO_2max) is not fully completed until 18 hours after the effort (LANDOR *et al.* 2002).

The main factors affecting blood rheology include erythrocyte deformability and aggregation, plasma viscosity, and the haematocrit index.

Blood rheology changes under the influence of effort. The impact of exercise on blood rheology depends on the type, duration, intensity, and on the athlete's individual characteristics. Some authors suggest a three-stage division of physical effort effects. Short-term effects of exercise include an increase in blood viscosity. Medium-term effects of regular exercise involve an increase in blood fluidity, which results from raised plasma volume (autoimmunization) and thus reduces both plasma viscosity and haematocrit level. Long-term effects improve blood fluidity, with simultaneous classical hormonal and metabolic changes caused by working out (BRUN *et al.* 1998).

Our hypothesis assumes that the long-term endurance efforts and exercise loads in triathlons may cause micro-injuries in amateurs, which intensify undesirable involution changes, up to death. BUBB (2012) notes that it is difficult to draw a line beyond which physical effort harms more than helps. Furthermore, he confirms that there is a risk of losing health benefits as a result of too much training, but this does not necessarily refer to each case. It seems thus obvious that gaining knowledge on how long and exhausting physical efforts change morphological, rheological, and biochemical blood indicators will help explain how the human body can adapt to such extreme efforts.

The aim of this paper was to assess how the impact of training- and competition-related factors changed morphological, rheological, and biochemical blood indicators during long-term effort in the Diablak Beskid Extreme Triathlon 2016 (the Carpathians, Poland), as well as the differences in these indicators between the triathletes and a control group (leading a sedentary lifestyle).

The following research question was posed: What is the range and direction of the changes in morphological, rheological, and biochemical blood indicators during long-term triathlon effort and what are the differences in these indicators between triathletes and a control group, leading a sedentary lifestyle?

Material and Methods

Study group

The study group comprised 10 male triathletes involved with the Active Side of Life Association; their mean age was 35 (30-45) years. The control group consisted of 10 males leading a sedentary lifestyle; their mean age was 35 (30-45) years (Fig. 1). The criteria for inclusion into the control group involved providing written informed consent to participate in the study, having a job related to many hours of work at a desk, a generally good health status, and being 30-45 years old. Chronic diseases, alcoholism, as well as nicotine and drug addiction constituted the exclusion criteria.

The mean training experience in the study group was 11±3.6 years, and the average sports level corresponded to the II and III sports class. The athletes trained over a single macrocycle. The training period was divided into three phases: basic phase, specialist phase, and immediate start preparation. Each phase was to prepare the competitors for the triathlon in a different way. During the first stage, the athletes worked out the technique of running, cycling, and swimming. The training was aerobic in its character. The aim of the specialist phase was to increase the working capacity during effort in the mixed zone and to gradually prepare the athletes for the later subthreshold work. Interval workouts focused on the pace of running and cycling were introduced. The third phase involved the improvement of swimming technique, interval training at the peri-threshold level, and specific workouts to prepare for the competition.

The athletes took part in the Beskid Extreme Triathlon 2016 (on June 19, 2016). The race was held in the Żywiec Beskids and the Silesian Beskids (the Carpathians, Poland). The competitors covered the following distances: 3.8 km of swimming, 180 km of cycling, and 44 km of running. The uphill stretches on the route constituted a difficulty during the race. The triathlon route started at 4:30 at Żywiec Lake, then led through Kubalonka, Salmopol Pass, and ended on Diablak (1725 m above sea level), the peak of Babia Góra, at the expected time of 18:00-20:00.

The triathletes' blood samples were collected before the competition (on June 17, 2016) and 36 hours after the competition (on June 21, 2016) in the Laboratory of Blood Physiology of the University of Physical Education in Krakow.

For the blood indicator analyses in the triathletes and in the control group, 5 ml of blood was collected from the ulnar vein, with no anticoagulant (clot tubes), into EDTA Vacuette tubes. The blood collecting procedure was performed after overnight fasting, by a qualified nurse in the morning, in accordance with the applicable standards of the Laboratory of Blood Physiology of the University of Physical Education in Krakow, where the morphological and rheological blood tests were carried out. The biochemical tests were done in the Department of Analytics and
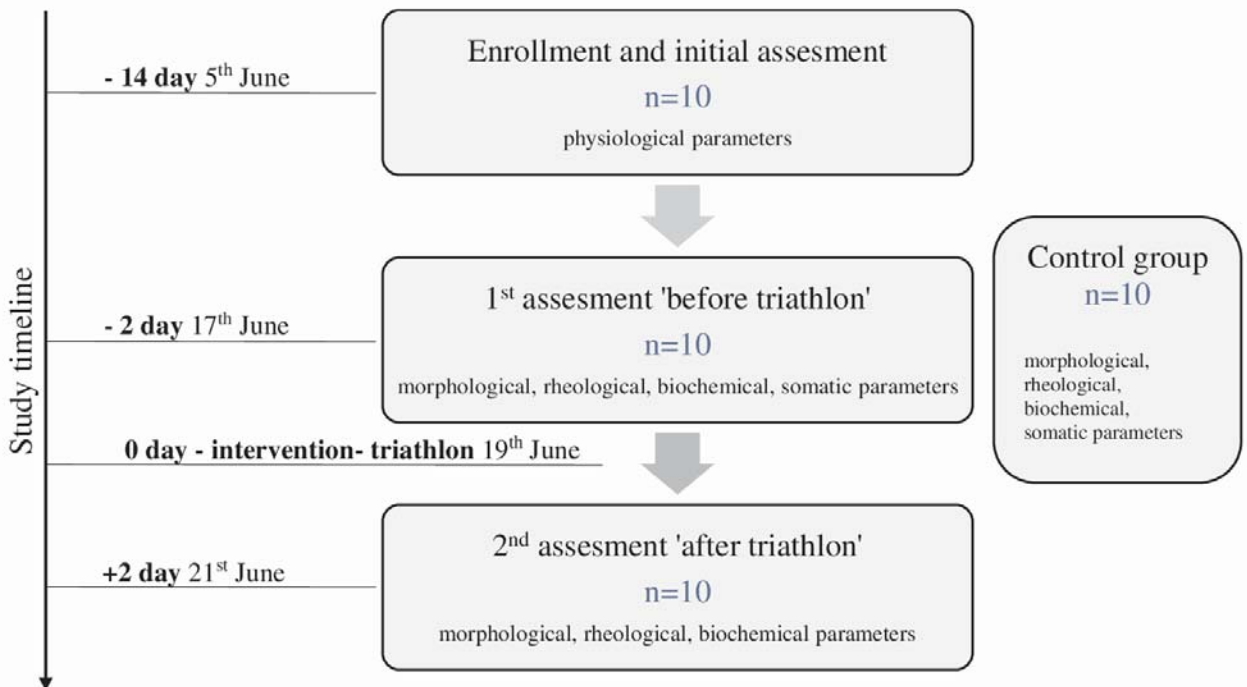

Fig. 1. Study design flowchart.

Clinical Biochemistry of the Kraków Oncology Centre. The study was approved by the Ethical Committee at the Regional Medical Chamber in Krakow (approval No.: 17/KBL/OIL/2015).

Somatic indicator assessment

Anthropometric measurements involved body height, body mass, fat mass, and lean body mass (Fig. 1).

Body mass and composition were determined by means of bioelectrical impedance with the use of a Jawon Medical, model IOI 353 (Korea) body composition analyser; body height was evaluated by a Martin (USA) antropometer, with a measurement accuracy of 1 mm. Fasting somatic indicators were assessed in the competitors and in the control group 2 days before the race.

Physiological indicator assessment

The physiological indicators were determined 2 weeks before participation in the DiablakBeskid Extreme Triathlon 2016 in order to assess the triathletes' training level (Fig. 1).

The assessment of maximal oxygen uptake (VO$_2$max) was performed with a graded treadmill test (Saturn 250/100R, h/p/Cosmos, Germany).

The effort started with a 4-minute warm-up at the speed of 8 km/h, with 1° ground inclination. Then, the running speed was increased by 1.1 km/h every 2 minutes. The test was continued until exhaustion.

During the test, the following indicators were recorded by using an ergospirometer (Cortex Meta-Lyzer R3): minute ventilation, percentage of carbon dioxide in exhaled air, oxygen uptake per minute, carbon dioxide production per minute, respiratory quotient, and ventilatory equivalent for carbon dioxide. The heart rate during the test was measured with a sports watch (Suunto Ambit 4, Finland).

Blood parameters measurements

Blood count was investigated in the ABX Micros 60 analyser.

As for blood rheology, the aggregation and deformability of red blood cells was tested with the Laser-assisted Optical Rotational Cell Analyser (LORCA). The Hardeman's method was used for the analysis and the results were presented with the elongation index (EI) (Hardeman *et al.* 2001).

To measure electrolytes, a Cobas device (Roche, Germany) was used with an ion-selective electrode (ISE) module to quantify potassium, sodium, and chloride ions. The following electrolytes were analysed: Na$^+$ (mmol/l) – concentration of sodium ions, K$^+$ (mmol/l) – concentration of potassium ions, Cl$^-$ (mmol/l) – concentration of chloride ions, total Ca^{2+} (mmol/l) – total calcium concentration, and Mg^{2+} (mmol/l) – concentration of magnesium ions.

For the renal and liver profile, the subjects' plasma was measured using a Roche/Hitachi Cobas c 311 haematological analyser. For each sample, the device automatically calculates the analytical activity of the given substance. The following blood biochemical indicators were measured in the renal profile: urea (mmol/l), creatinine (mmol/l), and uric acid (µmol/l). The following blood biochemical indicators were measured in the liver profile: total bilirubin (µmol/l), AspAT [U/l] – aspartate transaminase, AlAT (U/l) – alanine transaminase, GGT (U/l) – gamma-glutamyltransferase, and LDH (U/l) – lactate dehydrogenase.

Myocardial biochemical markers were assessed in Roche/Hitachi Cobas systems (creatine kinase and creatine kinase as a myocardial isoenzyme) and with the VIDAS® High sensitive test (troponin I). The following myocardial biochemical markers were caluated: CK (U/l) – creatine kinase, CK-MB (U/l) – creatine kinase, myocardial isoenzyme, and hs-Tn (ng/l) – high-sensitivity troponin.

The diagnostic indicators for determining immunoglobulins were assessed with a Dade Behring device in the BN ProSpec system. Reagents for *in vitro* diagnostics were designed to quantify immunoglobulins (IgG, IgA, IgM) in human serum with the immunonefelometric method. Protein electrophoresis was carried out in a Cobas c 311/511 analyser in Roche/Hitachi systems.

The concentration of 25(OH)D$_3$ (ng/ml), vitamin D metabolite, was determined by using DRG kits (DRG Instruments GmbH, Germany, test sensitivity: 0.1 µmol/l). Owing to post-workout dehydration, the values of biochemical indicators determined after triathlon completion were adjusted. The adjustment was made by first calculating %ΔPV from the formula described by Johansen *et al.* (1998). The formula developed by Kraemer and Brown (1986) was used to calculate the adjusted values.

Statistical analysis

An analysis of power was performed; the size of the study sample was calculated on the basis of 80% power and the value of $\alpha = 0.05$.

The data were presented as means and standard deviations or medians and interquartile ranges. The normality of distributions was verified with the Shapiro-Wilk test. The pre- and post-triathlon variables were compared with the Student's t-test for dependent variables, and, if the assumptions were not met, with the Wilcoxon test. The differences between the study group and the control group were analyzed with the t-test for independent variables, and, if the assumptions were not met, with the Mann-Whitney U test. The SS1/2 and EImax values were calculated by

matching the elongation curves to the Lineweaver-Burk model by using the non-linear matching algorithm in the GraphPad Prism 7.0 software (GraphPad Software Inc., La Jolla, CA, USA). The method was described in detail by BASKURT *et al.* (2009). A significance level of $\alpha = 0.05$ was assumed in the analyses. All p values were two-sided. The analyses were performed withStatistica 12 software (StatSoft®, USA).

Results

A detailed analysis of the somatic indices for the competitors and the control group is presented in Table 1.

Red blood cell elongation and aggregation indexes (Tables 4-6)

When analyzing the mean EI values in the study group (triathletes) before and after the triathlon and in comparison with the control group, statistically significant differences were found at the shear stress of 0.30 and 0.58 Pa (Table 4). It was demonstrated that the mean EI value increased after the triathlon and was different between the triathletes and the control group. There were no statistically significant differences for SS1/2 or SS1/2/EImax, which indexes no changes in erythrocyte deformability after the triathlon (Table 5). No statistically significant changes

Table 1

Selected somatic indices in the control and study groups

Group	BM (kg)	BH (cm)	LBM (kg)	FM (kg)	F% (%)
Competitors	76.3 ± 5.1	183.7 ± 3.1	64.8 ± 4.1	11.5 ± 3.5	15.1 ± 3.7
Control group	85.1 ± 3.5	185.3 ± 5.2	66.9 ± 2.9	18.2 ± 4.0	21.4 ± 4.6

BM – body mass, BH – body height, LBM – lean body mass, FM – fat mass, F% – percentage of fatty tissue.

Table 2 includes values of physiological indicators characterizing the maximum level of effort in the examined athletes.

Blood morphology (Table 3)

Before vs. after triathlon

The analysis of mean values of morphological indices in the study group (triathletes) before and after the triathlon showed a decrease in RBC (T/l), HGB (g/dl), and HCT (%) after the triathlon and a slight increase in MCV (fl).

Before triathlon vs. control group

In the group of the tested triathletes (before the triathlon), lower values of MCHC (g/dl) were found in comparison to the control group.

Table 2

Selected physiological indicators in the study group

Parameter	Maximum effort level
t (min)	19.0 ± 2.1
v (km/h)	15.9 + 1° ± 0.8
HR (beats/min)	179.4 ± 6.5
VO₂max (l/min)	4.2 ± 0.6
VO₂max (ml/kg/min)	55.0 ± 3.5
Ve (l/min)	157.9 ± 17.1
Distance (m)	3714.8 ± 240.1

t – time of work in the graded treadmill test, v – running speed, HR – heart rate, VO₂max – maximal oxygen uptake: globally (l/min) and with reference to body mass (ml/kg/min), Ve – minute ventilation.

Table 3

Mean values (± SD) of blood morphology indicators in the study group before and after the triathlon and in the control group

Parameter	Before triathlon (n = 10)	After triathlon (n = 10)	Control group (n = 10)	p (before-after)	p (before-control)
RBC (T/l)	4.45 ± 0.33	4.17 ± 0.23	4.65 ± 0.36	0.007*	0.171
HGB (g/dl)	13.53 ± 1.17	12.63 ± 0.88	13.69 ± 1.32	0.003*	0.518
HCT (%)	37.79 ± 2.86	35.59 ± 2.29	39.13 ± 3.48	0.008*	0.239
MCV (fl)	85.00 ± 2.24	85.56 ± 2.30	83.91 ± 1.92	0.013*	0.335
MCH (pg)	30.46 ± 1.39	30.21 ± 1.31	29.41 ± 0.90	0.179	0.080
MCHC (g/dl)	35.79 ± 0.78	35.44 ± 0.68	34.98 ± 0.65	0.169	0.033*
WBC (g/l)	4.82 ± 0.73	4.78 ± 0.82	5.42 ± 0.96	0.873	0.193
PLT (g/l)	184.67 ± 44.15	180.22 ± 32.92	187.00 ± 35.79	0.549	0.849

*significant difference (p<0.05);
RBC (T/l) – Red blood cells; HGB (g/dl) – Haemoglobin; HCT (%) – Haematocrit; MCV (fl) – Mean corpuscular volume; MCH (pg) – Mean corpuscular haemoglobin; MCHC (g/dl) – Mean corpuscular haemoglobin concentration; WBC (g/l) – Whiteblood cells; PLT (g/l) – Platelet count.

were observed for the other indexes: aggregation index (AI), AMP, or half-time of total aggregation (T1/2) (Table 6).

Blood biochemical indicators: electrolytes (Table 7)

Before vs. after triathlon

We found a statistically significant decrease of K^+ (by 5.5%), Ca^{2+}, and Mg^{2+}, and an increase in chloride levels (by 1.7%) after the triathlon. Sodium level changes did not present any statistical significance.

Before triathlon vs. control group

When comparing the group before triathlon with the control group, we observed statistically significant lower chloride levels (by 1.6%). The remaining changes did not present any statistical significance.

Renal function tests (Table 8)

Before vs. after triathlon

Intensive physical effort (running, cycling, swimming) decreased blood creatinine concentration (mmol/l) in the triathletes by 5.02%. In turn, no statis-

Table 4

Mean values (± SD) of red blood cell elongation indexes (EI) at various levels of shear stress in the study group before and after the triathlon and in the control group

Shear stress (Pa)	Before triathlon (n = 10)	After triathlon (n = 10)	Control group (n = 10)	p (before-after)	p (before-control)
0.30	0.000 ± 0.01	0.02 ± 0.03	0.01 ± 0.01	0.023*	0.047*
0.58	0.0 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.012*	0.007*
1.13	0.11 ± 0.01	0.13 ± 0.01	0.13 ± 0.02	0.068	0.113
2.19	0.22 ± 0.02	0.23 ± 0.01	0.22 ± 0.03	0.361	0.206
4.24	0.32 ± 0.02	0.33 ± 0.01	0.32 ± 0.03	0.820	0.674
8.23	0.39 ± 0.03	0.40 ± 0.02	0.40 ± 0.04	0.909	0.864
15.98	0.46 ± 0.04	0.47 ± 0.02	0.46 ± 0.04	0.939	0.864
31.03	0.51 ± 0.04	0.51 ± 0.04	0.50 ± 0.05	0.732	0.719
60.30	0.54 ± 0.04	0.54 ± 0.04	0.53 ± 0.05	0.595	0.642

*significant difference (p<0.05)

Table 5

Mean values (± SD) of red blood cell aggregation indexes (AI), half-time of total aggregation (T1/2), and total extent of aggregation (AMP) in the study group before and after the triathlon and in the control group

Parameter	Before triathlon (n = 10)	(After triathlon) (n = 10)	Control group (n = 10)	p (before-after)	p (before-control)
AI (%)	54.13 ± 8.57	57.14 ± 6.56	56.72 ± 9.18	0.820	0.573
T1/2 (s)	3.68 ± 1.35	2.97 ± 0.85	3.13 ± 1.51	0.287	0.170
AMP (au)	15.64 ± 3.92	18.25 ± 2.68	18.95 ± 4.49	0.149	0.079

Table 6

Mean values (± SD) of SS1/2, EImax, and SS1/2/EImax for red blood cells in the study group before and after the triathlon in the shear stress range of 0.3-60.30 (coefficient of determination for the model $R^2 > 0.99$)

Parameter	Before triathlon (n = 10)	After triathlon (n = 10)	p
SS1/2	4.08 ± 0.25	3.77 ± 0.29	0.07
EImax	0.58 ± 0.04	0.58 ± 0.02	0.78
SS1/2/EImax	7.05 ± 0.83	6.46 ± 0.52	0.16

EI values were measured for nine shear stresses between 0.3-60.30 Pa.

SS1/2 – the shear stress for half-maximal deformation

EI_{max} – maximum EI at infinite shear stress

Table 7

Mean values (± SD) of electrolyte concentrations in the study group before and after the triathlon and in the control group

Biochemical indicators	Before triathlon (n = 10)	After triathlon (n = 10)	Control group (n = 10)	p (before-after)	p (before-control)
Na^+ (mmol/l)	141.00 ± 1.50	140.11 ± 2.47	140.25 ± 1.29	0.249	0.216
K^+ (mmol/l)	4.52 ± 0.20	4.27 ± 0.25	4.32 ± 0.23	0.041*	0.176
Cl^- (mmol/l)	100.70 ± 1.47	102.39 ± 2.04	102.33 ± 1.39	0.009*	0.028*
Total Ca (mmol/l)	2.35 ± 0.04	2.29 ± 0.07	2.34 ± 0.09	0.027*	0.831
Mg^{2+} (mmol/l)	0.90 ± 0.07	0.85 ± 0.07	0.85 ± 0.06	0.003*	0.187

*significant difference (p<0.05)

tically significant changes were observed for urea (mmol/l) or uric acid (μmol/l).

Before triathlon vs. control group

No statistically significant differences were noted.

Liver function tests (Table 9)

No statistically significant differences were found for total bilirubin (μmol/l), AspAT (U/l), AlAT (U/l), GGT (U/l), or LDH (U/l) in the triathlete group before vs. after the triathlon, as well as in comparison with the control group.

Myocardial markers (Table 10)

Before vs. after triathlon

No statistically significant changes were found in the activity of CK (U/l) or CK-MB (U/l) when comparing the group of triathletes before and after the triathlon. After the triathlon, the concentration of hs-Tn (ng/l) increased by 25% compared with the initial value.

Before triathlon vs. control group

No statistically significant differences were observed.

Table 8

Mean values (± SD) of renal profile indicators in the study group before and after the triathlon and in the control group

Biochemical indicators	Before triathlon (n = 10)	After triathlon (n = 10)	Control group (n = 10)	p (before-after)	p (before-control)
Urea (mmol/l)	6.24 ± 1.27	6.40 ± 1.76	5.28 ± 1.37	0.575	0.060
Creatinine (mmol/l)	83.31 ± 10.06	79.33 ± 8.14	82.58 ± 10.98	0.006*	0.803
Uric acid (μmol/l)	317.97 ± 40.98	323.72 ± 323.72	314.82 ± 55.03	0.641	0.972

*significant difference (p<0.05)

Table 9

Mean values (± SD) or medians (IQR) of liver profile indicators in the study group before and after the triathlon and in the control group

Biochemical indicators	Before triathlon (n = 10)	After triathlon (n = 10)	Control group (n = 10)	p (before-after)	p (before-control)
Total bilirubin (μmol/l)	11.01 ± 3.63	10.82 ± 4.26	9.98 ± 4.75	0.844	0.455
AspAT (U/l)	20.10 (18.90-31.00)	30.00 (22.10-52.70)	20.8 (16.9-24.2)	0.260	0.413
AlAT (U/l)	23.84 ± 8.06	26.64 ± 10.45	21.77 ± 9.82	0.219	0.570
GGT (U/l)	22.30 (15.20-24.90)	22.40 (15.50-27.70)	23.5 (15.85-34.25)	0.441	0.859
LDH (U/l)	180.09 ± 52.37	184.94 ± 49.00	161.21 ± 23.78	0.595	0.644

Table 10

Medians (IQR) of myocardial profile indicators in the study group before and after the triathlon and in the control group

Biochemical indicators	Before triathlon (n = 10)	After triathlon (n = 10)	Control group (n = 10)	p (before-after)	p (before-control)
CK (U/l)	141.50 (111.55-230.70)	142.0 (139.90-365.90)	177.95 (116.3-266.66)	0.213	0.972
CK-MB (U/l)	13.80 (10.90-14.10)	13.10 (12.90-18.40)	10.95 (9.4-13.1)	0.767	0.135
hs-Tn (ng/l)	1.50 (1.50-1.50)	2.00 (1.50-3.80)	1.5 (1.5-1.5)	0.043*	0.861

*significant difference (p<0.05), CK (U/l) – creatine kinase, CK-MB (U/l) – creatine kinase, myocardial isoenzyme, hs-Tn (ng/l) – high-sensitivity troponin.

Immunoglobulins (IgG, IgA, IgM), protein electrophoresis, C-reactive protein (Table 11)

Before vs. after triathlon

When comparing the group before and after the triathlon, the following were found: decrease in total protein (g/l) (by 4.6%), decrease in albumin (g/l) (by 4.72%), decrease in alpha-2-globulin (g/l) (by 5.56%), decrease in beta-2-globulin (g/l) (by 6.08%), decrease in gamma-globulin (g/l) (by 6.64%), increase in % alpha-1-globulin (g/l) (by 8.62%), increase in % beta-1-globulin (g/l) (by 4.42%), decrease in IgG (g/l) (by 5.61%), decrease in IgA (g/l) (by 11.81%), decrease in IgM (g/l) (by 16.67%), increase in C-reactive protein (CRP) (mg/l) (by 245%).

Before triathlon vs. control group

No statistically significant differences were observed.

Serum levels of the $25(OH)D_3$ metabolite

No statistically significant changes were found when comparing the values before (46.79±6.55 ng/ml) and after (47.93±5.64 ng/ml) the triathlon.

Table 11

Mean values (± SD) or medians (IQR) of selected serum protein parameters in the study group before and after the triathlon and in the control group.

Biochemical indicators	Before triathlon (n = 10)	After triathlon (n = 10)	Control group (n = 10)	p (before-after)	p (before-control)
Total protein (g/l)	70.40 ± 1.89	67.16 ± 3.83	70.69 ± 5.33	0.030*	0.776
Albumin (g/l)	44.07 ± 1.41	41.99 ± 2.22	44.30 ± 3.23	0.037*	0.915
Alpha-1-globulins (g/l)	2.46 ± 0.24	2.53 ± 0.17	2.54 ± 0.27	0.357	0.445
Alpha-2-globulins (g/l)	5.76 ± 0.79	5.44 ± 0.65	5.79 ± 0.52	0.037*	0.887
Beta-1-globulins (g/l)	3.82 ± 0.35	3.82 ± 0.50	4.16 ± 0.40	1.000	0.062
Beta-2-globulins (g/l)	3.62 ± 0.60	3.40 ± 0.60	3.42 ± 0.57	0.040*	0.498
Gamma-globulins (g/l)	10.70 ± 1.74	9.99 ± 1.94	10.54 ± 2.17	0.006*	0.943
A/G (g/l)	1.67 ± 0.11	1.67 ± 0.11	1.69 ± 0.16	1.000	0.972
% Albumin (g/l)	62.59 ± 1.52	62.56 ± 1.56	62.68 ± 2.26	1.000	0.943
% Alpha-1-globulins (g/l)	3.48 ± 0.37	3.78 ± 0.37	3.62 ± 0.49	0.033	0.454
% Alpha-2-globulins (g/l)	8.08 ± 1.20	8.16 ± 1.30	8.20 ± 0.73	0.719	0.568
% Beta-1-globulins (g/l)	5.43 ± 0.44	5.67 ± 0.58	5.88 ± 0.51	0.025*	0.093
% Beta-2-globulins (g/l)	5.12 ± 0.76	5.04 ± 0.69	4.83 ± 0.69	0.133	0.354
% Gamma-globulins (g/l)	15.20 ± 2.42	14.80 ± 2.30	14.79 ± 2.21	0.002*	0.749
IgG (g/l)	11.41 ± 1.61	10.77 ± 1.98	11.13 ± 2.29	0.022*	0.696
IgA (g/l)	2.88 ± 0.93	2.54 ± 0.95	2.31 ± 0.66	0.001*	0.154
IgM (g/l)	0.60 (0.50–0.70)	0.50 (0.50–0.60)	0.95 (0.65–1.35)	0.028*	0.211
CRP (mg/l)	0.40 (0.28–0.49)	1.38 (1.22–7.71)	0.51 (0.199–1.05)	0.021*	0.776

*significant difference (p<0.05)

Discussion

Among the studies published so far, there are no reports that would address the impact of triathlons on morphological, rheological, and biochemical blood indicators in a comprehensive and standardized way. Recently, a paper presenting changes in the markers of muscle and intestinal damage in athletes training for triathlons appeared (TOTA *et al.* 2019); however, it did not analyze such a wide range of indicators as this study.

Blood morphology

Physical effort considerably impacts plasma volume. Blood plasma volume decreases by ca. 15% during exercise and increases after the effort. Plasma volume rises owing to fluid supplementation, reduced kidney water and sodium excretion, and water migration from tissues to vessels. There is also a phenomenon known as rhabdomyolysis, which occurs when skeletal muscle cells are damaged during intensive physical effort. As a result of the damage, myoglobin, enzymes, and electrolytes, among others, are released to the blood. Excessive concentrations of these substances can lead to the damage of some organs, particularly the kidneys. In advanced cases pain, oedema, and muscle fatigue are observed, and urine becomes darker as a result of myoglobinuria, i.e. the presence of myoglobin particles in the urine. Rhabdomyolysis can also remain asymptomatic. Athletes undergoing intensive physical exercise and untrained individuals are at the highest risk (WOJTASIK *et al.* 2015).

In a study by LIU *et al.* (2018), 19 runners were examined who participated in the Ultramarathon in Taipei. No statistically significant changes were observed for RBC, HGB, or HCT. In turn, in our study, a decrease in RBC, HGB, and HCT was found in the group of subjects after the triathlon. The decrease in RBC resulted from post-effort haemolysis, which consists of a reduction of erythrocyte count with simultaneous haemoglobin passage into blood plasma. Red blood cell deformation raises the risk of their decomposition. This can be caused by increased body temperature, which is inevitable during intensive physical effort (WOJTASIK *et al.* 2015). Another reason for post-workout haemolysis may be a too-low blood glucose concentration (hypoglycaemia). It is a temporary phenomenon in athletes, resulting from inadequate carbohydrate intake before intensive physical effort. The decrease in erythrocyte count in the investigated triathletes may also be associated with excessive iron loss. One of the triathlon disciplines is long-distance running, which also affects changes in erythrocyte count. Regular and prolonged hitting of the foot on the ground causes the destruction of the red blood cells in the blood vessels of the lower extremities (DĄBROWSKI 1998; VAN WIJK & VAN SOLINGE

2005). The reduction in haemoglobin concentration may be caused by haemolysis – a mechanism of destroying erythrocytes during and after physical activity (BÖNING *et al.* 2007; SUHR *et al.* 2009). Furthermore, a decrease in haemoglobin values may result from haemodilution, a phenomenon related to endurance training, which appears in athletes due to increased plasma volume (NEUMAYR *et al.* 2005).

Many publications imply that excessive blood viscosity impairs performance in endurance disciplines, inhibiting microcirculation and oxygen supply to muscle cells (SCHUMACHER *et al.* 2000; WIRNITZER & FAULHABER 2007). The present study confirms the favourable effect of increasing mean corpuscular volume (MCV) 36 hours after a triathlon.

High values of certain blood morphotic components are essential for achieving an optimal aerobic capacity level. Erythrocytes are of particular importance, but the mean corpuscular haemoglobin concentration (MCHC) is also crucial, as evidenced by a higher MCHC value before the triathlon in comparison with the control group.

Blood rheology

Intensive physical effort in triathletes during the Diablak Beskid Extreme Triathlon did not lead to changes in red blood cell deformability or aggregation (AI, AMP, T1/2).

In our study, no statistically significant changes in erythrocyte deformability at the shear stress above 0.58 Pa were found in the triathletes, as evidenced by the SS1/2 and SS1/2/EImax indexes when comparing the triathlete group before and after the triathlon and the triathlete group with the control group. Statistically significant differences in mean EI values were observed at the shear stress of 0.30 and 0.58 Pa only when analyzing the study group (triathletes) before and after the triathlon and when comparing the study group with the control group. No statistically significant differences were found for AI, AMP, or T1/2. This is most likely due to the athletes' physical fitness. The lack of deterioration in erythrocyte deformability after the triathlon observed in our research is undoubtedly a positive effect of proper body conditioning. Recent reports indicate that physical effort reduces or increases erythrocyte deformability. A possible link between the beneficial metabolic and hemodynamic effects of exercise could be blood rheology, which is markedly affected by exercise. Short-term effects of exercise are an increase in blood viscosity resulting from both fluid shifts and alterations of erythrocyte rheologic properties (rigidity and aggregability). Increased blood lactate, stress, and acute phase play a role in this process. Middle-term effects of regular exercise are a reversal of these acute effects with an increase in blood fluidity, explained by plasma volume

expansion (autohemodilution) that lowers both plasma viscosity and hematocrit. Long-term effects further improve blood fluidity, parallel with classical training-induced hormonal and metabolic alterations. While body composition, blood lipid pattern, and fibrinogen improve (thus decreasing plasma viscosity), erythrocyte metabolic and rheologic properties are modified, with a reduction in aggregability and rigidity. The destroyed deformability of red blood cells that occurs during prolonged physical effort with high oxygen consumption results from oxidative stress, caused by the production of free radicals originating in the mitochondria, leukocytes, or from temporary tissue hypoxia. During exercise, an increase in erythrocyte stiffness is observed, followed by a gradual return to the initial state. Another reason for destroyed deformability is an increase in lactate (an anaerobic glycolysis product) concentration above 4 mmol/l. Hormones such as glucagon and noradrenaline, as well as leukotrienes B4 and C4 decrease erythrocyte deformability during physical effort; the atrial natriuretic peptide increases deformability. Changes in deformability may be caused, among others, by disturbances in the properties of the erythrocyte membrane. The enormous effort to which athletes are subjected (such as in triathlons) may also affect the physicochemical properties of the erythrocyte environment (pH level, hydrostatic pressure, amphipathic substances); these modifications may considerably impact erythrocyte deformability. Changes in the shape of erythrocytes are also dependent on cytoplasm viscosity, which is determined by the intracellular concentration of haemoglobin. The above-mentioned properties of red blood cells are also influenced by the geometry of microvessels, mechanical characteristics of the cytoskeleton (BRUN *et al.* 1998, 2000, 2010). Changes in blood rheological properties have been observed in overtrained athletes. BRUN *et al.* (2010) revealed that individuals with a heavy leg feeling and signs of overtraining presented a higher plasma viscosity index and a higher erythrocyte aggregation index. This research implies that a heavy leg feeling in overtrained athletes results from haemorheological disorders. In another study, BRUN *et al.* (1998) implied that in overtrained individuals, haematocrit levels and plasma viscosity increased and ferritin concentration decreased. No changes in erythrocyte aggregation or deformability were demonstrated. According to BILSKI *et al.* (2014), blood viscosity increases after physical effort, which is accompanied by an increase in haematocrit, a decrease in erythrocyte deformability, and an increase in red blood cell aggregation. Additionally, a meal eaten after exercise results in a further decrease in blood cell deformability and an increase in erythrocyte cell membrane stiffness. The authors point out that the above haemorheological changes should not impair the cardiovascular function in healthy, fit individuals, but may pose a health hazard in various pathophysiological conditions.

Electrolytes

The physical effort applied by the triathletes during the Diablak Beskid Extreme Triathlon caused a decrease in K^+, Ca^{2+}, and Mg^{2+} ions and an increase in Cl^- ions; no statistically significant changes were demonstrated for Na^+ ions. The raised Cl^- ion concentration after the competition most probably indicates an additional hydration of the body during the exercise and after the competition. In turn, the Cl^- ion values were higher in the study group before triathlon than in the control group, although these changes remain within the normal range for Cl^-. No statistically significant changes for Na^+ ions may be associated with the body hydration during the triathlon competition. These results are in line with those obtained by KNECHTLE *et al.* (2010) in a group of 27 triathletes participating in the Ironman distance, who did not present any statistically significant changes, and with a study by HEW-BUTLER *et al.* (2006). The reduction of K^+, Ca^{2+}, and Mg^{2+} ions in triathletes after competition depends on the type and amount of fluids that they drink during the race.

When comparing the group before the triathlon with the control group, we observed statistically significantly lower chloride levels (by 1.6%) in the latter. The remaining differences did not present any statistical significance.

Renal and liver profiles

The presented study revealed a statistically significant decrease in creatinine (by 5.02%) in the study group after participation in the Diablak Beskid Extreme Triathlon. This was most likely caused by the 36-hour break after the intensive triathlon and indicates normal kidney function. It is known that effort results in a short-term increase in serum creatinine concentration, mainly owing to an increased metabolism of phosphocreatine consumed for energy purposes. This is supported by PUGGINA *et al.* (2014), who observed a statistically significant increase in creatinine among 12 triathletes when comparing results before and after completion of a triathlon. The authors explained that this was most likely due to the influence of exercise on glomerular membrane permeability and the secretion of antidiuretic hormones, aldosterone, or catecholamines. For the remaining biochemical indicators (urea, uric acid, total bilirubin, AspAT, AlAT, GGT, LDH), no statistically significant changes were found, which may indicate that the triathletes were used to this type of effort. No statistically significant differences were noted for renal or liver profiles between the athletes before the triathlon and the control group.

Myocardial markers

Biochemical markers of myocardial damage are used in clinical diagnostics, mainly after myocardial infarction or surgery. The increase in a given marker concentration is proportional to the degree of myocardial damage (THOMPSON *et al.* 1996). However, it is reported that even with muscle damage markers present in blood, there appear no disorders in muscular function (MAYERS & NOAKES 2000; NEUBAUER *et al.* 2008).

Intensive physical effort increased troponin I blood concentration in the triathletes by 25%. Blood biochemical markers (CK, CK-MB) did not exhibit statistically significant differences in the group of triathletes before and after the triathlon or in comparison with the control group. In their research on CK, KOCH *et al.* (2014) identified several groups that reacted with different blood CK release under the influence of physical effort. As they mention in their study, researchers disagree as to the qualification of subjects as NR (normal response), LR (low response), or HR (high response) (KOCH *et al.* 2014). According to CLARKSON (1992), the qualification thresholds are values of 500 U/l for the LR group, between 500 and 2000 U/l for the NR group, and over 2000 U/l for the HR group. The absence of statistically significant changes allows for the presumption that the athletes from the Active Side of Life Association are eligible for the LR group. KOCH *et al.* (2014) also suggest that regular workouts and familiarization with a given activity reduce CK release after exercise. The Diablak Beskid Extreme Triathlon was held at the end of the group's training season, which may imply their good training level. As expected, no increase in CK activity was observed in our study.

The obtained results stand in contrast to previous research. The difference may result from the sampling time (over 24 hours after the triathlon completion). Studies performed among athletes taking part in Olympic distance triathlons confirm the effects observed in the research on individual disciplines. In a study by LOPES *et al.* (2011), CK activity increased after each stage, and the biggest increase was found after cycling. It exceeded the normal reference values already before the start; the activity decreased 1 hour after the race, but still maintained a level indicating muscle damage. In our research, neither the group of triathletes before or after the competition nor the control group presented CK activity exceeding normal values. To be considered diagnostically significant, the CK-MB marker level must reach 6% of the total CK value (KLEIN *et al.* 2001). Research implies that an increase in CK-MB activity is expected after a race, but does not lead to any long-lasting consequences in an athlete's body (MAYERS & NOAKES 2000). This is confirmed by studies investigating CK-MB activity and electrical myocardial disorders. They did not reveal any positive correlation between

the performed tests. Increased CK-MB activity does not reflect myocardial disorders (LA GERCHE *et al.* 2004; VLECK *et al.* 2014). A study by LOTT and STANG (1980) on swimmers in a training cycle showed only minimal amounts of CK-MB, insufficient to qualify the event as myocardial damage. In turn, WOLF *et al.* (1987) state that athletes are characterized by naturally elevated CK-MB activity (higher than in non-training people), which may be caused by continuous hypoxia conditions. At the same time, with the increased CK-MB activity, the electrocardiogram remained unchanged.

The measurement of troponin I and T concentration has now become the gold standard in the diagnosis of myocardial infarction. Because of the wide availability of the test, troponin measurements are often performed in athletes practising endurance sports, such as marathons, ultramarathons, triathlons, etc. The concentration of hs-Tn is sustained up to 5-15 days after they enter the bloodstream (STĘPIEŃ *et al.* 2002). In a study by SHAVE *et al.* (2010), the participants were to run on a treadmill continuously for 30 minutes (the effort during the exercise was considerably lower in comparison with Ironman-type competitions); the hs-Tn concentration increased in 75% of the individuals. In a recent study on cold water swimming, the subjects swam a distance of their choice: 500 m, 750 m, or 1000 m. The distance was shorter than that in a typical triathlon, but the conditions were different from those usually faced on a triathlon route (in winter triathlons, skiing is substituted for swimming). The researchers observed a statistically significant increase in hs-Tn concentration in all participants (BROŽ *et al.* 2017). As with swimming, investigation on cycling shows an increase in hs-Tn concentration after a race. The troponin concentration reaches its peak about 14-20 hours after the effort (STĘPIEŃ *et al.* 2002). Other scientists report similar results. At the same time, they indicate that the post-workout raise of troponins persists in the blood for a shorter time than that following myocardial infarction (SCHARHAG *et al.* 2008). Elevated values of troponin I concentration are also noted in marathon participants. Often, troponin I concentrations exceed those characteristic of myocardial infarction. Such values were measured among participants of marathons in Mainz, Maas, Leadville, Boston, and Perth, among others. As with the other triathlon components, the observed concentration of troponin I is raised, which seems to indicate that the results after the triathlon will also be higher (FORTESCUE *et al.* 2007; HERRMANN *et al.* 2003; HUBBLE *et al.* 2009; KHODAEE *et al.* 2015; KLEIN *et al.* 2001). In our study, troponin I concentration after the completed triathlon increased by 25% on average compared with the values before the race (this is a statistically significant change). This corroborates research on the individual triathlon component disciplines; however, studies performed among triathletes reveal an opposite trend: troponin I concentration is

slightly raised or undetectable. These results can be found, among others, in papers by LA GERCHE *et al.* (2004), RAMA *et al.* (1996), and RIFAI *et al.* (1999). Still, not all causes of this phenomenon have been identified. It can perhaps be attributed to the high dynamics of troponin I concentration changes resulting from physical effort (RAMA *et al.* 1996; SCHARHAG *et al.* 2008). If the test after triathlon completion was performed 24 hours later, it is likely that troponin I concentration would have returned to the pre-contest value.

Proteins related to the immune system

Proteins are the basic structural material in the body, involved in enzymes, receptors, hormones, and antibodies; they transport various chemical compounds. Intense physical effort affects the proteins of the immune system in triathletes.

In our research, statistically significant changes in some of the immune system protein indicators were found when comparing the study group before and after the triathlon. A decrease was reported in the following indicators: total protein (g/l), albumin (g/l), alpha-2-globulin (g/l), beta-2-globulin (g/l), gamma-globulin (g/l), % alpha-1-globulin (g/l), IgG (g/l), IgA (g/l), and IgM (g/l). In turn, for CRP (mg/l) and % beta-1-globulin (g/l), increased values were observed.

However, no statistically significant differences were observed between the study group before the triathlon and the control group.

A low concentration of protein fractions and immunoglobulins 36 hours after the triathlon as compared with the group before the triathlon indicates the presence of acute inflammation in the triathletes. In our study, the immunoglobulin level decreased, which might raise the risk of upper respiratory tract infections. GLEESON and PYNE (2000) also pointed out that very intense exercise could inhibit IgA secretion, while moderate exercise could exert positive effects (MACKINNON 2000). The authors observed that IgA and IgM concentrations dropped immediately after intensive training, but usually returned to normal within 24 hours. They claim that high-intensity training applied over many years can chronically inhibit immunoglobulin secretion. The immunity decrease and recovery rate after exercise are related to exercise intensity, or the training duration or volume. Low IgM and IgA concentration predisposes to upper respiratory tract infections.

In the presented study, an increase in CRP by as much as 245% was observed in the study group after the triathlon, which indicates the body's response to the effort. These results remain in line with a study by NEUBAUER *et al.* (2008), who also reported an increase in inflammation markers in 42 well-trained endurance athletes. In a study by DEL COSO *et al.* (2014), athletes who covered the triathlon distance in a longer time presented much higher concentrations of inflammation indicators.

Serum levels of the 25(OH)D₃ metabolite

Vitamin D occurs as a so-called prohormone, or a hormone precursor, which becomes an active enzyme only after a suitable modification. The active form of vitamin D, responsible for the multi-level importance of vitamin D in the human body, is $1,25(OH)_2D_3$, while the most common serum concentration measurement refers to the inactive form, $25(OH)D_3$. Vitamin D is increasingly considered an important metabolite in the proper functioning of muscles both in training individuals and in elderly physically inactive people (GUNTON & GIRGIS 2018). The normal serum concentration of $25(OH)D_3$ has a beneficial effect on the muscular system during and after physical activity as it reduces inflammation. This is a consequence of maintaining appropriate concentrations of pro- and anti-inflammatory cytokines (TNF-α and interleukin 10). Through the activation of intracellular receptors, vitamin D supports protein synthesis in muscle cells, maintains adequate ATP levels, improves physical performance (strength, mass, endurance), reduces delayed onset muscle soreness, has a protective effect on type II fast-twitch muscle fibres, and accelerates post-workout regeneration (TRAUTVETTER *et al.* 2014). Our study did not reveal any statistically significant changes in the serum concentration of $25(OH)D_3$.

Conclusions

The extreme physical activity undertaken by athletes participating in the Diablak Beskid Extreme Triathlon 2016 allowed us to determine the changes in the morphological, rheological, and biochemical blood indicators in triathletes. The results obtained from the triathletes imply that the parameters that should be assessed with special care in the context of health risk include blood morphological and rheological indicators, proteinogram, electrolyte concentrations, and cardiac profile indicators.

Blood haematologic and biochemical indicators in individuals participating intriathlons characterize the actual range and direction of effort-related changes well and allow for the diagnosisof transient adaptive effects. Rheological parameters, involving the evaluation of erythrocyte deformability and aggregation, are useful for monitoring the particularly undesirable, short- and long-term effects of practicing extreme sports such as participating in triathlons. The assessment of complex blood biochemical and rheo-

logical parameters allows for individualized and multidirectional interventions in the exercise-associated period and thus for appropriate training and then regeneration of the body after extreme physical effort such as participation in a triathlon.

Multisport disciplines such as triathlons are becoming increasingly popular worldwide. However, many people practicing these intense activities are not fully aware of their possible harmful effects. The performed study illustrates the change of body parameters after intense physical effort. The results support the significance of controlling the morphological, biochemical, and rheological blood parameters in competitive athletes, including triathletes.

Acknowledgments

Author Contributions

Conflict of Interest

The authors declare no conflict of interest.

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