Abstract

Purpose: to study the aspects of organism’s cardio-hemo-dynamic and blood erythrocytes reaction of female volleyball players to standard physical load.

Material: with functional methods we studied cardio-hemo-dynamic and with the help of scanning electronic microscopy – erythrocytes’ structure in 18 female volleyball players of different qualification (age – 22.0±0.60 years).

Results: it was found that maximal physical load causes substantial changes in cardio-hemo-dynamic, which depend on female volleyball players’ qualification. These changes have intrinsic to them type of blood circulation system reacting, which is manifested in the following: appropriate changes of some indicators; natural changes of periphery blood erythrocytes. In the article possible mechanisms of realization of female volleyball players’ organism’s typological features, depending on blood circulation type and erythrocytes’ conformation, are discussed.

Conclusions: In relaxed state all female volleyball players have non-uniform cardio-hemo-dynamic of blood circulation. With hyper-dynamic blood circulation type, higher indicators of strike and minute blood volume were observed. With hypo-kinetic blood circulation type the opposite picture was observed: indicators of strike and minute blood volume, heart index, load on cardio-vascular system in different periods of day were low.

Keywords: female volleyball players, blood circulation, heart index, physical load, erythrocytes.

Introduction

The level of human organism’s responding to physical load illustrates health condition and ability to sustain workability in professional functioning. For such assessment different approaches are used, selection of which depends on category of population. For assessment of students’ reaction to physical load appropriate tests for motor activity are used [24, 50]. The tests application facilitates the following: optimization of physical loads [19, 30]; correction of training process [37, 52]; improvement of motor skills [29, 41]; proper organization of pedagogic control [28, 36].

For determination of athletes’ reaction to physical loads other approaches are used. Their aim is finding of the following: level of motivation to sports practicing [16, 43]; successfullness in sports [20, 31]; individual approaches to training [22, 25]; working out of training models [21, 27].

Among such approaches proper place is taken by tests, based on finding of cardio-hemo-dynamic indicators. Such approaches are frequently used in sports. It permits to find athletes’ reaction to physical loads and correct the process of preparation for competitions.

By the range of heart index (HI) oscillations central cardio-anaerobic (CHD) is divided into hypokinetic (HK), eukinetic (EK) and hyper-kinetic (HrK) types of blood circulation. They are variants of hemo-dynamic norm [1, 3, 5].

Physical load of maximal intensity (PLmax) can result in CHD changes and create pre-conditions for disorder of erythrocyte homeostasis [4, 12, 13]. With it, CHD has definite typological features and depends on quickness of system blood circulation’s reacting. In such case changes of periphery blood erythrocytes (EPB) with PLmax are determined by bio-chemical reactions. Such reactions lead to reduction of acid/alkaline balance. In its turn, acidosis causes deformation of EPB [46].

Reactions of athletes’ organisms to maximal and standard physical loads are shown in the following directions:

− Study of blood bio-chemical indicators and their influence on health of 100 meters’ runners [33];
− Finding of energy supply mechanisms of athletes’ high workability by blood bio-chemical indicators [17];
− Finding of physical effects of football referees’ workability by lactate content in blood [18];
− Quantitative assessment of training load in team kinds of sports (testing on run track for quickness and lactate concentration in blood). The authors found that individualized impulse training can be used for improvement of aerobic indicators in competition season [45];
− Distribution of professional mini-football athletes’ load. The research of blood bio-chemical indicators permitted to find mechanisms of physical workability improvement [48];

Adaptation to physical loads permitted to determine:

− Mechanisms of muscular strength and muscular mass age reduction, connected with chronic inflammation. Physical activity causes anti-inflammation effect, but it is modulated by additional factors [26];
− Regular physical exercises facilitate reduction

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of oxidation stress and cause some changes in metabolism of iron. These changes were connected with reduction of blood ferritine. The authors offer the program of Nordic walking (12 weeks, thrice a week) [38];

- Nordic Walking reduces resistance to insulin [40];
- Nordic Walking significantly reduces ferritine concentration in blood that explains the registered reduction of iron in organism [39].

As some specialists point non uniformity of CHD types is conditioned by body constitution and is a physiological norm of health [2, 6, 9]. Study of EPB changes with PL_{max} depending on CHD type is still out of scientists’ attention. Which correlations of different EPB conformation forms in athletes with different blood circulation hemodynamic type has not been clear [2, 13]. The data of some authors point at same type of organism’s reaction to hemodynamic type has not been clear [2, 13]. The data of other authors [7, 8] – prevails one of them [4, 7, 10]. The question about role of EPB in origin of such reactions is still unsolved [8, 11, 13]. Studies of CHD types in children resulted in idea that they are genetically determined [12]. But not equal percentage of these types in different age groups contradicts to it [3, 9, 14]. With it, the question about special aspects of CHD and EPB reactions to single standard physical load, depending on athletes’ sportsmanship and CHD type still remains to be out of specialists’ attention [6, 8].

**Hypothesis:** it was assumed that athletes with different CHD type have different organism’s sensitivity to standard physical load, that is manifested as different conitative EPB changes.

The purpose of the work is to study the aspects of organism’s cardio-hemo-dynamic and blood erythrocytes reaction of female volleyball players to standard physical load, depending on qualification level.

**Material and methods**

**Participants:** 18 girl students of 20.0±0.6 yrs age, who practiced volleyball, participated in the research. Depending on girl students’ qualification, they were divided into two groups. First group consisted of 11 girl students, who attend volleyball training, but have no sport category. Group 2 included female volleyball players of supreme league team (masters of sports) – members of team of Vasyl Stefanyk Precarpathian National University.

**Organization of the research:** application of PL_{max} of 3.5 W/kg of body mass is the most optimal methodic for ergo metric testing (ET) of different age athletes’ physical workability [12-14]. For determination of CHD indicators we used ergo meter Kettler (Germany) and computer diagnostic complex “CardioLab+”. Cardio-hemo-dynamic mechanisms of short term adaptation to PL_{max} we assessed by indicators HI; systolic (SBP) and diastolic (DBP) blood pressure; average blood pressure (ABP). By changes of heart beats rate we found: stroke blood output (SBO); minute blood output (MBO). By value of load on cardio-vascular system in different periods of day (PD) we determined energetic characteristics of heart functioning and myocardium demands in oxygen [14]. It is calculated by formula: PD = (BP:HBR)/100, where BP is blood pressure and HBR – heart beats rate.

For studying erythrocytes’ conformation and biochemical characteristics we took capillary blood by protocol of determination of glucose concentration in blood plasma. Taking of material was fulfilled just before PL_{max} and after 1-3 minutes of recreation period. Concentration of hemoglobin was tested by standard method. The quantity of erythrocytes was tested by unified method of calculation in Goryaev’s chamber. Hematocrit was found by micro-method with application of standard heparinized capillaries. Morphological studies of erythrocytes were carried out with the help of scanning electronic microscope «JEOL-25M-T220A» (Japan) by commonly accepted methodic [23]. All studies were fulfilled at the end of academic year.

**Statistical analysis:** the received quantitative indicators were processed with the help of STATISTICA 6.0 program.

**Results**

The studies showed that in relaxed state volleyball players of both groups (see tables 1, 2) have non uniform CHD different types.

Typological analysis [2, 9, 11] showed that 42% of the tested female volleyball players have hypo-kinetic, 31% – eukinetic and 27% – hyper-kinetic type of CHD.

In volleyball players of 2^{nd} group with HrBC type we observed confidently higher indicators of HBR, SBO, MBO, HI and PD (p<0.05). In case of HBC type initial values were the lowest. EBC indicators were at average level. In relaxed state, in 2^{nd} group female volleyball players with HrBC type indicators SBO, MBO and HI were high (p<0.05) and PD indicator was low. In relaxed state PD indicator took intermediate position between hyper and hypo-kinetic types of CHD.

PL_{max} was followed by changes in hemo-dynamic of different expressiveness. These changes have certain typological specificities of blood circulation’s reacting. In tables 1 and 2 we can see that PL_{max} from the first minute causes noticeable increase of SBP in both groups’ volleyball players with different CHD. ABP is substantially changed after first minute of load. HBR became maximal up to the end of the fifth minute of PL_{max}. HBR increased depending on CHD: accordingly, 2.27; 2.18 and 2.14 times (P<0.05). Concerning 1^{st} group volleyball players with he same reactions of blood circulation to PL_{max}, their HBR was the lowest (accordingly by 9.26%, 10.38% and 14.77%; p<0.05).

Thus, increase of SBP under PL_{max} in 1^{st} group took place at the account of SBO increasing. It is known that increase of SBO results in reciprocal inhibition of sinus node automation. It reduces HBR [3, 9]. It is observed in 1^{st} groups volleyball players with blood circulation of different type.

PL_{max} in 1^{st} group volleyball players with HrBC causes insignificant (in average by 5.3±0.11%, P <0.05) increase of EPB. In 2^{nd} group there happens statistically significant
increase of EPB. It is facilitated by hemo-concentration (15% volleyball players with HBC). In 85% girl students with EBC it causes reduction of their quantity. It is conditioned by destructive influence of factors, which accompany muscular activity. It is observed with weakening of cardio-respiratory system’s adaptation potentials. They are: increased blood circulation; rising of temperature and acidosis [21].

In contrast to 1st group (see fig. 1a) in 2nd group’s volleyball players with HBC there appear some reversibly changed forms of EPB (see fig. 1b). In girls students with EBC erythrocytes remain unchanged after PLmax (see fig. 2a, b). Fig.1 Structural reconstruction of periphery blood erythrocytes in 1st (a) and 2 (b) groups’ volleyball players with hypo-kinetic hemo-dynamic type after single maximal physical load. Legend: 1 – normal forms of erythrocytes; 2 - reversibly changed forms of erythrocytes; 3 – irreversibly changed forms of erythrocytes. The method is – scanning electronic microscopy, scale: 1500:1.

In 2nd group’s volleyball players with HrBC we observed increase of EPB aggregation ability (content of aggregates increased by 15%) and increase of coefficient of their deformation (by 18%). It is a result of muscular work (see fig. 3).

Discussion
The conducted earlier studies showed [11, 53], that at the end of academic year female volleyball players of 2nd group have low level of functional reserves. It requires more careful attention of different specialization scientists to students’ physical condition, who practice intensive trainings in parallel to main studies.

PL of different intensity plays great role in formation of organism’s general endurance. Such endurance is especially required for the following: in different competitions; in trainings; in everyday life of students-athletes [42, 53]. In such case EPB is a convenient object for such researches: they participate in processes, connected with sustaining of the whole organism’s homeostasis [34, 44, 49]. In our research we found that in 57% of 2nd group’s volleyball players, under influence of PLmax negative morphological changes of EPB appear. It is conditioned by dis-metabolic disorders. The basis

| Table 1. Indicators of different types of cardio-hemo-dynamic of 1st group’s volleyball players before and after standard physical load and in recreation period (M±m, n=7) |
|---|---|---|---|---|---|
| Indicators | Blood circulation type | relaxed state | 1 min | 3 min | 5 min | Recreation time |
| HBR, bpm | HBC | 71,8±2,3 | 118,3±2,8* | 144,8±3,4* | 150,1±4,8* | 108,3±4,2* | 89,1±3,2 |
| | EBC | 75,8±2,5 | 120,7±2,6* | 149,0±3,2* | 152,1±4,5* | 115,9±4,3* | 94,8±2,1 |
| | HrBC | 85,0±3,1 | 124,0±3,4* | 134,4±4,1* | 147,1±4,4* | 114,0±3,1* | 95,0±2,6 |
| SBP, mm/merc.col | HBC | 117,0±3,2 | 144,0±3,2* | 158,1±4,3* | 170,1±5,2* | 135,1±4,2* | 116,9±3,2 |
| | EBC | 118,0±2,1 | 151,9±2,9* | 164,4±3,2* | 178,9±4,3* | 134,0±3,7* | 120,1±4,7 |
| | HrBC | 126,0±1,1 | 160,6±2,6* | 168,0±3,3* | 179,0±4,6* | 139,0±3,4* | 123,2±3,6 |
| DBP, mm/merc.col | HBC | 60,0±1,2 | 65,0±1,4 | 72,0±2,1 | 76,0±1,4 | 73,0±1,6 | 70,1±1,2 |
| | EBC | 65,0±1,4 | 70,0±1,7 | 73,0±1,4 | 74,0±1,2 | 68,0±1,1 | 67,3±1,6 |
| | HrBC | 67,0±1,2 | 68,0±1,1 | 74,0±1,5 | 77,0±1,9 | 68,0±1,6 | 66,0±1,1 |
| ABP mm/merc.col | HBC | 88,5±3,4 | 106,5±4,1* | 115,0±4,5* | 123,1±4,5* | 104,2±3,2* | 93,5±2,3 |
| | EBC | 91,5±2,3 | 112,3±3,3* | 118,7±3,6* | 126,3±5,9* | 101,0±4,3* | 93,7±2,6 |
| | HrBC | 96,0±2,1 | 114,3±4,2* | 121,0±3,3* | 128,5±5,4* | 103,5±3,8 | 94,6±2,1 |
| SBO, ml | HBC | 4,2±0,4 | 6,4±0,5* | 7,7±0,9* | 8,5±1,2* | 5,6±0,3* | 4,6±0,5 |
| | EBC | 5,3±0,3 | 7,8±0,4* | 8,7±1,1* | 10,2±1,8* | 6,7±0,4* | 5,3±0,5 |
| | HrBC | 7,2±0,4 | 10,6±0,7* | 11,9±1,4* | 12,9±2,1* | 8,8±0,9 | 7,2±0,6 |
| MBO, l | HBC | 4,2±0,1 | 4,8±0,3* | 9,4±0,4* | 6,8±0,3* | 3,3±0,2* | 2,8±0,1 |
| | EBC | 3,4±0,2 | 5,2±0,4* | 7,0±0,6* | 7,8±0,4* | 4,1±0,4* | 3,5±0,2 |
| | HrBC | 4,3±0,4 | 7,8±0,6* | 8,1±0,8* | 8,6±0,7* | 4,8±0,5 | 4,1±0,5 |
| HI, l/min/m | HBC | 79,8±2,2 | 150,7±8,3* | 209,4±10,2* | 223,9±11,1* | 140,9±12,7* | 82,0±6,2 |
| | EBC | 88,9±2,7 | 201,7±9,6* | 233,9±11,2* | 245,4±14,2* | 145,1±15,6* | 91,6±6,7 |
| | HrBC | 109,9±3,2 | 211,8±10,2* | 244,3±12,3* | 252,7±15,6* | 166,3±16,1* | 106,4±6,8 |

Notes: 1) HBC – hypokinetic blood circulation; EBC – eukinetic blood circulation; HrBC – hyperkinetic blood circulation; 2) * – difference is confident at р<0.05. Absence of mark means that difference is not confident. In all cases confidence was found in comparison with relaxed state.
Table 2. Cardio-hemo-dynamic indicators of 2nd group’s volleyball players with different blood circulation type before physical load and in recreation period (M±m, n=11)

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Blood circulation type</th>
<th>Relaxed state</th>
<th>Duration of physical load</th>
<th>Recreation time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 min</td>
<td>3 min</td>
<td>5 min</td>
</tr>
<tr>
<td>HBR, bpm</td>
<td>HBC</td>
<td>72,1±2,3</td>
<td>122,9±2,1*</td>
<td>145,5±2,7*</td>
</tr>
<tr>
<td></td>
<td>EBC</td>
<td>77,0±2,2</td>
<td>122,3±2,4*</td>
<td>148,1±5,0*</td>
</tr>
<tr>
<td></td>
<td>HrBC</td>
<td>79,3±2,5</td>
<td>140,9±2,3*</td>
<td>153,0±3,1*</td>
</tr>
<tr>
<td>SBP, mm/merc.col</td>
<td>HBC</td>
<td>107,8±2,3</td>
<td>136,4±3,1*</td>
<td>151,0±3,3*</td>
</tr>
<tr>
<td></td>
<td>EBC</td>
<td>111,8±2,1</td>
<td>140,2±3,8*</td>
<td>153,6±4,3*</td>
</tr>
<tr>
<td></td>
<td>HrBC</td>
<td>115,5±2,2</td>
<td>143,2±3,0*</td>
<td>156,2±3,4*</td>
</tr>
<tr>
<td>DBP, mm/merc.col</td>
<td>HBC</td>
<td>65,2±3,1</td>
<td>60,3±2,1</td>
<td>61,1±2,2</td>
</tr>
<tr>
<td></td>
<td>EBC</td>
<td>69,8±2,2</td>
<td>70,3±2,4</td>
<td>71,6±3,9</td>
</tr>
<tr>
<td></td>
<td>HrBC</td>
<td>71,0±3,3</td>
<td>74,6±4,1</td>
<td>80,7±4,3</td>
</tr>
<tr>
<td>ABP mm/merc.col</td>
<td>HBC</td>
<td>86,5±2,7</td>
<td>98,4±2,6*</td>
<td>106,1±2,7*</td>
</tr>
<tr>
<td></td>
<td>EBC</td>
<td>90,8±2,1</td>
<td>105,3±3,1*</td>
<td>112,6±4,1*</td>
</tr>
<tr>
<td></td>
<td>HrBC</td>
<td>93,3±2,7</td>
<td>108,9±3,5*</td>
<td>118,5±3,9*</td>
</tr>
<tr>
<td>SBO, ml</td>
<td>HBC</td>
<td>47,4±2,2</td>
<td>67,8±3,1*</td>
<td>64,3±3,2*</td>
</tr>
<tr>
<td></td>
<td>EBC</td>
<td>55,9±2,3</td>
<td>78,9±2,3*</td>
<td>77,8±2,4*</td>
</tr>
<tr>
<td></td>
<td>HrBC</td>
<td>72,1±2,3</td>
<td>97,2±3,3*</td>
<td>90,1±3,1*</td>
</tr>
<tr>
<td>MBO, I</td>
<td>HBC</td>
<td>3,9±0,3</td>
<td>7,1±0,5*</td>
<td>8,2±0,3*</td>
</tr>
<tr>
<td></td>
<td>EBC</td>
<td>4,8±0,5</td>
<td>8,3±0,4*</td>
<td>8,7±0,6*</td>
</tr>
<tr>
<td></td>
<td>HrBC</td>
<td>6,2±0,3</td>
<td>9,7±0,5*</td>
<td>11,4±0,7*</td>
</tr>
<tr>
<td>HI, l/min/m</td>
<td>HBC</td>
<td>2,3±0,4</td>
<td>4,2±0,3*</td>
<td>5,3±0,3*</td>
</tr>
<tr>
<td></td>
<td>EBC</td>
<td>3,0±0,2</td>
<td>5,4±0,5*</td>
<td>6,0±0,3*</td>
</tr>
<tr>
<td></td>
<td>HrBC</td>
<td>3,9±0,5</td>
<td>8,7±0,8*</td>
<td>8,9±0,5*</td>
</tr>
<tr>
<td>PD, conv.un.</td>
<td>HBC</td>
<td>76,3±1,2</td>
<td>167,9±12,3*</td>
<td>225,8±34,4*</td>
</tr>
<tr>
<td></td>
<td>EBC</td>
<td>86,1±1,8</td>
<td>180,7±15,1*</td>
<td>241,7±23,2*</td>
</tr>
<tr>
<td></td>
<td>HrBC</td>
<td>99,6±3,6</td>
<td>209,4±16,7*</td>
<td>248,9±24,3*</td>
</tr>
</tbody>
</table>

Notes: 1) HBC – hypokinetic blood circulation; EBC – eukinetic blood circulation; HrBC – hyperkinetic blood circulation; 2) * – difference is confident at р<0.05. Absence of mark means that difference is not confident. In all cases confidence was found in comparison with relaxed state.

of such changes is exhaustion of functional potentials of organism’s bio-systems. It is facilitated by negative influence of combined increased mental and physical load during academic year [53].

The peculiarity of 2nd group volleyball players’ with HBC hemo-dynamic reacting to PLmax was active involvement of mechanism of periphery blood circulation regulation mechanism in work. It increases local blood flow at the account of widening of working muscles’ vessels.

The main mechanism of ABP sustaining for HeBC is high values of HI. They are determined by contracting ability of left ventricle with low values of total periphery resistance (TPR) of vessels.

Heart works in ineffective mode that is why its compensatory potentials are limited. For such type high activity of sympathetic-adrenaline system is characteristic. In case of HBC, in homeostasis sustaining tonus of hemo-vessels of blood systems artery part dominates. In our research we observed high TPR, but power of left ventricle’s contraction is minimal [1, 3, 13]. This type of blood circulation is the least effective and has low adaptation potential [12, 47].

As a number of authors notes [2, 11, 13], organism of people with different blood circulation type reacts to PLmax by increasing of HI: from hypo-kinetic to hyper-kinetic type. Independent on group, in volleyball players with HrBC we observed the highest HI indicators under PLmax. However, comparing with relaxed state they increase only 1.79 times in 1st group and 2.46 times in 2nd group (р<0.05).

The received data show that in relaxed state, the presence of EBC and HrBC set high requirements to energy supply of heart functioning.

Intensive external heart work (especially in 1st group’s volleyball players with HrBC) can be explained prevailing of SBP indicators, which is accompanied by increase of myocardium demand in oxygen [1, 3, 7]. With high myocardium demand in oxygen [1, 3, 7] there is high ratio of oxygen in myocardium of 2nd group’s girl students we observed higher absolute values of such indicators as: heart work; energy consumption for transportation of one liter of MBO; power of left
ventricle’s contraction and stroke output quickness.

Under such conditions damage of EPB structural wholeness appears. It facilitates their in-vessels’ lysis and can result in progressing of anemia [46]. Such changes negatively influence on somatic health, sport efficiency and educational progress of students. It requires appropriate correction of educational and training process. Besides, it is necessary to liquidate negative after-effects. It is important to work out and use in due time adequate measures, directed at removal of possible pathological changes in organisms of students-athletes.

In opinion of some authors [49], EPB changes facilitate increase of viscosity of circulating blood. It will negatively influence on CHD type and saturation of tissues with oxygen. In the whole such bio-chemical and conformation changes of EPB are very unfavorable for realization of oxygen/transportation function of blood. They witness about insufficient organism’s adaptation of

![Fig. 1](image1.png) Structural reconstruction of periphery blood erythrocytes in 1st (a) and 2 (b) groups’ volleyball players with hypo-kinetic hemo-dynamic type after single maximal physical load. Legend: 1 – normal forms of erythrocytes; 2 - reversibly changed forms of erythrocytes; 3 – irreversibly changed forms of erythrocytes. The method is – scanning electronic microscopy, scale: 1500:1.

![Fig. 2](image2.png) The structure of periphery blood erythrocytes of 2nd group’s volleyball players with eukinetic blood circulation type after single maximal physical load. The method is – scanning electronic microscopy, scale: a -1400:1 and b - 1500:1.
2nd group’s volleyball players (especially with HrBC to PL\textsubscript{max} at the end of academic year).

We have found interconnection between sportsmanship of volleyball players after PL\textsubscript{max} by CHD type. It points at demand in its value’s gradation by quantitative-qualitative changes of EPB. Such approach can be used as a criterion for determination of adequate volume of training loads during academic year. It is in agreement with analogous criteria, found by other authors [32].

\textbf{Conclusions}

1. All tested female volleyball players in relaxed state demonstrate cardio-hemo-dynamic non uniformity of blood circulation. With hyper-kinetic blood circulation type we observed higher indicators of stroke and minute blood output and index of load on cardio-vascular system in different periods of day. With hypo-kinetic type of blood circulation’s regulation we observed the opposite picture: indicators of stroke and minute output, heart index and load on cardio-vascular system in different periods of day were low.

2. In the process of 2nd group’s volleyball players’ with HBC adaptation to maximal physical load we observed standard increase of stroke blood output already in the first minute of muscular functioning. With it periphery part of blood circulation’s regulation was actively involved in work.

3. In case of eukinetic blood circulation type to larger extent cardio-hemo-dynamic reacting to maximal physical load manifested. In 1st group’s volleyball players’ indicators of systolic blood pressure and load on cardio-vascular system in different periods of day reached significant values. In 2nd group’s volleyball players indicators of minute blood output were higher. In 1st group intensification of external work of heart was realized at the account of high systolic blood pressure. In 2nd group it happened due to increase of minute blood output.

4. In 1st group’s volleyball players with hyper-kinetic blood circulation type, under maximal physical load we observed significant increase of systolic blood pressure and stroke blood output. In 2nd group, since the 1st minute stroke blood output increased noticeably. With it, in the work of periphery blood circulation’s regulation we observed weakening of functional reserves. It was illustrated by increased quantity of deformed erythrocytes; by reduction of their size; increase of “adhesion threads” and small erythrocyte aggregates.

\textbf{Conflict of interests}

The authors declare that there is no conflict of interests.
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