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Original Article

# The Experimental Study on the Alterations of Hemodynamics and Mechanical Characteristics of Erythrocyte Membrane after Different Training

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**ABSTRACT**

**Background and Aim:** The effect on erythrocyte membrane after different training and recovery, by evaluating relationship between the changes of mechanical characteristics of erythrocyte membrane and hemodynamic indices was assessed. **Materials and Methods:** The Sprague Dawley rats were divided into 5 groups (n=7). The rats were trained on treadmill for four weeks, six days per week except for control group that stayed sedentary. The effect of training on hematocrit and mechanical characteristics of the erythrocyte membrane (elasticity and interaction energy) by using dielectrical electrophoresis and the mathematical model were assessed. **Results:** Training for 3 weeks improved hematocrit and mechanical characteristics after recovery and elasticity and interaction energy in RBC membrane decreased transitorily at different levels after high intensity training. **Conclusion:** The effect of training in RBC mechanical characteristics is that changes of RBC membrane protein after high-intensity training cause the changes in RBC membrane structure and hence changes hemodynamics.

**Keywords:** Red Blood Cell Membrane, Mechanical Property in membrane, Training, Hemodynamics, Sprague Dawley rats

## INTRODUCTION

Training hemorheology has been the subject of many studies in athletes in sedentary people and patients suffering from various diseases.[1-3] Recently, we developed concepts aimed at bringing together all this information in order to provide an integrated view of both the mechanical characteristics of erythrocyte membrane and the functional consequences of the hemorheological alterations observed during and after training.

In most cases (e.g. short acute training), these hemorheological variables explain virtually all the observed increase in whole blood viscosity.[4] When post training (e.g. recovery) values are measured, these short-term alterations (hematocrit etc.) were probably not detected, due to a rapid return to pre-training values.[2-3] In most (but not all) training protocols there are changes in the rheological properties of RBC. The most classical is a decrease in RBC deformability which is not a specific finding since it is also observed in most stressful event like labor, video film-induced emotional stress, and endogenous depression.[5] These effects are generally not found at training when RBC rheology is investigated after resuspension of cells in a buffer, indicating that they are mostly due to plasma factors rather than to intrinsic RBC properties.[2,4] Blood lactate, which experimentally shrink the RBC and decreases their flexibility, is likely to explain in pair this training-induced rigidification of RBC, as supported by positive correlations blood lactate concentrations and RBC rigidity at training. Interestingly, in one study we found a threshold value for this effect which became apparent only when blood lactate increased above 4 mmol·1-1, i.e. a value which has been proposed to represent approximately the point where lactate induces acidosis.[6] However, some other studies suggest that lactate also exerts some effects at lower concentrations, either *in vivo* or *in vitro*.[1,5,7] Lactate is not the only factor explaining this rigidification. Traumatic damage of RBC due to their compression in the foot plantar circulation is likely to be important in sports like running, although this issue remains unclear.[3]

Presumably, fluid status has also a major influence on RBC rheology during training, as suggested by the preventive effect of fluid intake on RBC rigidification.[8] There is also an acute increase in RBC aggregation and disaggregation shear rate.[9] While RBC rigidity was generally found to be either increasing or unchanged during training.[1,2,6,8,10,11] Thus, in highly trained subjects, the training-induced increase in blood lactate does not rigidity the RBC as observed in sedentary subjects or in moderately trained ones (like soccer players) but actually improves RBC deformability.[6] Theoretically, most of the rheologic

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changes reviewed above are likely to exert negative effects on training performance.

Training-induced changes in blood rheology have been reporter to be related to the rating of perceived exertion. Increased hematocrit (Hct) positively correlated with perceived exertion.[12] And, during a subsequent standardized submaximal training test, the runner exhibited a disproportionate increase in Hct and RBC aggregation and disaggregation thresholds. However, it is noteworthy that we observed the same rheological changes during submaximal training as those during maximal workloads.[10] This leads us to propose that simple changes in Hct, RBC rigidity and ηP are physiological adaptive modifications which occur during many kinds of training and do not imply a risk by themselves.

Dielectrophoresis (DEP) is a well-known method for manipulation of dielectric particles such as DNA, protein and cells.[13] This specific electrokinetic technique has been used for trapping, sorting, focusing, filtration, pattering, assembly, and separating biological entities/particles suspended in a buffer medium.[9] DEP is defined as the translation motion as a result of the polarization induced by electric fields.[14] With this method, cells can be manipulated to higher and lower electric field regions by means of polarization forces that induce what is named as positive and negative DEP.[13] The electrophoretic force is dependent on the dielectric properties and size of the cell, frequency of the electric field and permittivity and conductivity of the medium that the cells are suspended.[15] In this study, DEP was applied to evaluate the mechanical properties (elasticity and interactive energy) of RBCs membrane based on its chain formation, confirming the alterations of hemodynamics and mechanical characteristics of erythrocyte membrane after different training in rats.

## MATERIALS AND METHODS

### Equipment

1. Hematocrit (HAEMATOCRIT 210, Germany)
2. Generator (TESLA)
3. Microscope (10R)
4. Treadmill (MK-680AT/02R)

### Reagents

1. RBC diluting fluid (D.P.R. Korea)
2. 5% Sodium citrate (D.P.R. Korea)
3. Castor sugar (D.P.R. Korea)

### Methods

#### *Measurement of the mechanical properties of RBC membrane using DEP*

DEP is a method that has demonstrated great potential in cell discrimination and isolation.[16] Using this method, the cells manipulated to the highest electric field regions become safe when they contact closely each other in the same direction that a linking line of cells' centers is coincident with the one of electric field due to the minimum energy. At this time, the red blood cells form a chain by means of dipole-dipole interaction.[17] In our study, the chain is formed between electrodes when a drop of erythrocyte suspension is placed in the electrophoretic and flow room. Then, the speed controller is controlled so that the chain is formed. When the suspension (external force) is experienced to the chain of erythrocyte, the chain is extended and the size of erythrocyte is changed. The mechanical properties (K: elasticity, C: capacity, E: interactive energy) of RBC membrane are calculated from following formula:

$$K(l-l_o)+\left(\frac{C\bullet V^2}{2N^2}+E\right)\frac{\pi}{2}\bullet\frac{\Delta^2(l)}{\sqrt{l^2+\frac{2S_o}{\pi}}}-\frac{fd^2}{8\delta}=0 \quad (1)$$

where $\ell_0$ is the primary length of RBC, $\ell$ is the extended length of RBC, N is the number of erythrocytes in a line chain, $V$ is the external applied voltage, δ is the length of chain and $d$ is the distance between electrodes. Here, $f$ is an external force that is applied at per length of columnar RBC whose diameter is △, described by:

$$\cdots\cdots f=4\pi\eta v/\ln(7.4\eta/\rho v\Delta) \quad (2)$$

where η is the viscosity and ν is the flow velocity of liquid. In addition, the surface area of erythrocyte, $S_0$, is calculated by following formula:

$$S_0=\pi\Delta^2/2+\pi\Delta\ell \quad (3)$$

The coefficients of K, C, E are obtained in three different situations. For K, C and E, it is, firstly, made three chains that are set in the range of three effective voltages amplitude, and measured the flow speed of suspension that is needed for bending the chain arcuatedly to values that is corresponding value of the first (6.7μm), second (13.4μm) and third (20.1μm) scale. Putting these values into eqn. (1), and we can be obtain following equations, then, using diagonal law, the values of K, C and E of RBC membrane can be calculated from these equations.

#### *Subjects and training methods in experimental group*

The Sprague Dawley rats weighed 110~230g were randomly divided into 5 groups. Sedentary group (control), low intensity training group (LT), 24 hr recovery after LT group (LT24), high intensity training group (HT), 24 hr recovery after HT group (HT24). Each group had seven rats. The rats were trained on treadmill for four weeks, six days per week except for control group that stayed sedentary. Training in the first two weeks was to accustom the rats to the training session, the training intensity increased 5m/min with the beginning load of 15m/min, until it reached 30m/min, Group LT were trained 20 min every day, from the third week, the training was extended to 30 min. Group HT were trained 20 min every day, from the third week, the training was extended to 30 min. The former group was resting for 24 hr (LT24 and HT24). We observed the changes of hct as a hemodynamic index and the changes of the mechanical characteristics of the erythrocyte membrane (elasticity and interaction energy) by using dielectrical electrophoresis and the mathematical model.

## RESULTS

Table 1 showed hematocrit in LT and HT trained with control group and group trained for 30min a day during 4 weeks increased, comparing with control group and group trained for 15min ($P < 0.05$, $< 0.01$). All the recovery groups showed insignificant hematocrit than control group.

Table 2 showed the membrane elasticity in LT and HT trained for 25min, 30min a day during 3 weeks decreased, comparing with CON and group trained for 15min ($P < 0.05$, $< 0.01$). All the recovery groups showed insignificant elasticity than CON.

Table 3 showed the interaction energy in RBC membrane in LT and HT trained for 25min, 30min a day during 4 weeks increased, comparing with CON and group trained for 15min ($P < 0.05$, $< 0.01$). All the recovery groups showed insignificant elasticity than CON.

Table 4 showed the membrane elasticity in LT and HT trained for 25min, 30min a day during 4 weeks decreased, comparing with CON and group trained for 15min ($P < 0.05$,$< 0.01$). All the recovery groups showed significant elasticity than CON and group trained for 15min.

**Table 1: The changes of hematocrit in LT and HT trained for 4 weeks and recovery for 24 hr.**

| Groups | Time (min) | | | |
|---|---|---|---|---|
| | 15 | 20 | 25 | 30 |
| CON | 52±1.1 | 50±1.3 | 56±2.1 | 53±2.1 |
| LT | 52±2.5 | 50±2.3 | 60±1.3 | 62±2.5*$^{\Delta}$ |
| HT | 52±2.4 | 52±2.3 | 66±2.0 | 68±1.8**$^{\Delta\Delta}$ |
| LT24 | 53±1.3 | 52±2.3 | 54±1.5 | 53±2.1 |
| HT24 | 50±2.0 | 52±2.3 | 55±1.3 | 54±2.5 |

*$^{\Delta}$Significant difference ($P$<0.05); **$^{\Delta\Delta}$Significant difference ($P$<0.01)

*comparison with CON; $^{\Delta}$comparison with group trained for 15min

**Table 2: The changes of elasticity in LT and HT trained for 3 weeks and recovery for 24 hr.**

| Groups | Time (min) | | | |
|---|---|---|---|---|
| | 15 | 20 | 25 | 30 |
| CON | 2.73±0.28 | 2.97±0.10 | 3.00±0.11 | 2.76±0.10 |
| LT | 2.86±0.31 | 2.79±0.09 | 2.13±0.10*$^{\Delta}$ | 1.86±0.11**$^{\Delta}$ |
| HT | 2.81±0.26 | 2.38±0.08 | 1.99±0.10**$^{\Delta\Delta}$ | 1.71±0.12**$^{\Delta\Delta}$ |
| LT24 | 2.70±0.25 | 3.18±0.13 | 2.78±0.10 | 3.05±0.09 |
| HT24 | 2.58±0.21 | 2.78±0.12 | 2.76±0.10 | 2.78±0.10 |

*$^{\Delta}$Significant difference (P<0.05); **$^{\Delta\Delta}$Significant difference (P<0.01)

*comparison with CON; $^{\Delta}$ comparison with group trained for 15min

**Table 3: The changes of interaction energy in RBC membrane trained for 3 weeks and recovery for 24 hr.**

| Groups | Time (min) | | | |
|---|---|---|---|---|
| | 15 | 20 | 25 | 30 |
| CON | 1.11±0.05 | 1.17±0.05 | 1.15±0.04 | 1.08±0.05 |
| LT | 1.13±0.05 | 1.18±0.06 | 1.30±0.05*$^{\Delta}$ | 1.35±0.07**$^{\Delta\Delta}$ |
| HT | 1.18±0.05 | 1.20±0.07 | 1.32±0.06*$^{\Delta}$ | 1.40±0.07**$^{\Delta\Delta}$ |
| LT24 | 1.13±0.04 | 1.03±0.05 | 1.05±0.06 | 1.10±0.07 |
| HT24 | 1.10±0.08 | 1.09±0.07 | 1.10±0.08 | 1.08±0.06 |

*$^{\Delta}$Significant difference (P<0.05); **$^{\Delta\Delta}$Significant difference (P<0.01)

*comparison with CON; $^{\Delta}$comparison with group trained for 15min

**Table 4: The changes of elasticity of RBC membrane trained for 4 weeks and recovery for 24 hr.**

| Groups | Time(min) | | | |
|---|---|---|---|---|
| | 15 | 20 | 25 | 30 |
| CON | 2.76±0.23 | 2.98±0.10 | 2.96±0.11 | 2.86±0.10 |
| LT | 2.97±0.31 | 2.66±0.09 | 2.00±0.07*$^{\Delta}$ | 1.74±0.12**$^{\Delta\Delta}$ |
| HT | 2.81±0.21 | 2.22±0.09 | 1.90±0.10**$^{\Delta\Delta}$ | 1.68±0.12**$^{\Delta\Delta}$ |
| LT24 | 2.85±0.19 | 2.70±0.09 | 2.09±0.06*$^{\Delta}$ | 1.88±0.05*$^{\Delta}$ |
| HT24 | 2.75±0.17 | 2.60±0.08 | 2.00±005*$^{\Delta}$ | 1.73±0.05*$^{\Delta}$ |

*$^{\Delta}$Significant difference (P<0.05); **$^{\Delta\Delta}$Significant difference (P<0.01)

*comparison with CON; $^{\Delta}$comparison with group trained for 15min

**Table 5: The changes of interaction energy in RBC membrane trained for 4 weeks and recovery for 24 hr.**

| Group | Time(min) | | | |
|---|---|---|---|---|
| | 15 | 20 | 25 | 30 |
| CON | 1.10±0.05 | 1.09±0.07 | 1.14±0.05 | 1.12±0.06 |
| LT | 1.12±0.05 | 1.21±0.09 | 1.32±0.04**$^{\Delta}$ | 1.36±0.06**$^{\Delta\Delta}$ |
| HT | 1.13±0.05 | 1.24±0.09 | 1.39±0.04**$^{\Delta}$ | 1.42±0.08**$^{\Delta\Delta}$ |
| LT24 | 1.17±0.06 | 1.10±0.06 | 1.20±0.06 | 1.18±0.07 |
| HT24 | 1.19±0.07 | 1.12±0.07 | 1.20±0.07 | 1.20±0.07 |

*$^{\Delta}$Significant difference ($P$<0.05); **$^{\Delta\Delta}$Significant difference ($P$<0.01)

*comparison with CON; $^{\Delta}$ comparison with group trained for 15min

Table 5 showed the interaction energy in RBC membrane in LT and HT trained for 25 min, 30 min a day during 4 weeks increased, comparing with CON and group trained for 15 min (P<0.05,<0.01). All the recovery groups showed insignificant elasticity than CON.

## DISCUSSION

Both maximal and submaximal training, either of long duration, almost always increases blood viscosity due to a rise in plasma viscosity and hematocrit. In most cases (e.g. short acute training), these two hemorheological variables explain virtually all the observed increase in whole blood viscosity.[4] Table 1 showed hematocrit in LT and HT trained with CON and group trained for 30min a day during 4 weeks increased, comparing with CON and group trained for 15min (P <0.05,<0.01). All the recovery groups showed insignificant hematocrit than CON. This rise in ηp and Hct is sometimes interpreted as hemoconcentration.[17] In most training models there are changes in the rheological properties of RBC. The most classical is a decrease in RBC deformability which is not a specific finding since it is also observed in most stressful event like labor, video film-induced emotional stress, and endogenous depression.[5] These effects are generally not found at training when RBC rheology is investigated after resuspension of cells in a buffer, indicating that they are mostly due to plasma factors rather than to intrinsic RBC properties.[2,4]

Presumably, fluid status has also a major influence on RBC rheology during training, as suggested by the preventive effect of fluid intake on RBC rigidification.[8]

Table 2 showed the membrane elasticity in LT and HT trained for 25min, 30min a day during 3 weeks decreased, comparing with CON and group trained for 15min (P <0.05,<0.01). All the recovery groups showed insignificant elasticity than CON. Table 3 showed the interaction energy in RBC membrane in LT and HT trained for 25min, 30min a day during 4 weeks increased, comparing with CON and group trained for 15min (P <0.05,<0.01). All the recovery groups showed insignificant elasticity than CON. There is also an acute increase in RBC aggregation and disaggregation shear rate.[2] *In vitro* experiments[18] showed that lactate at concentrations ranging from 2 to 10mM increased RBC deformability in such athletes while it classically decreased it in blood from sedentary subjects.

Table 4 showed the membrane elasticity in LT and HT trained for 25min, 30min a day during 4 weeks decreased, comparing with CON and group trained for 15min (P <0.05,<0.01). All the recovery groups showed significant elasticity than CON and group trained for 15min. Table 5 showed the interaction energy in RBC membrane in LT and HT trained for 25min, 30min a day during 4 weeks increased, comparing with CON and group trained for 15min (P <0.05,<0.01). All the recovery groups showed insignificant elasticity than CON. Training-induced

changes in blood rheology have been reporter to be related to the rating of perceived exertion. Increased Hct positively correlated with perceived exertion[12] and was hypothesized to represent a signal among the other well-characterized ones (e.g., heart rate, lactate, blood glucose) that are integrated at a conscious level to generate the feeling of exertion. This leads us to propose that simple changes in Hct, RBC rigidity and ηP are physiological adaptive modifications which occur during many kinds of training and do not imply a risk by themselves. Rheological effects may be responsible in part for the enhanced incidence of myocardial infarction an sudden death associated with training.[19-21]

## CONCLUSION

Long-term training can increase hematocrit effectively and elasticity and interaction energy in RBC membrane decreased transitorily at different levels after high intensity training. Training for 3 weeks can improve hematocrit and mechanical characteristics after recovery. One possible mechanism of trainings effect in RBC mechanical characteristics is that changes of RBC membrane protein after high-intensity training cause the changes in RBC membrane structure and hence changes hemodynamics.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**RBC:** Red Blood Cell; **Hct:** Hematocrit; **DEP:** Dielectrophoresis; **LT:** Low Intensity Training; **HT:** High Intensity Training; **CON:** Control.

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